

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. *(Currently amended)* A method for detecting ketosteroids, comprising:

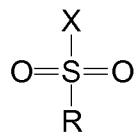
reacting a sample with a sulfonylhydrazide to form a sulfonylhydrazone of a ketosteroid in the sample;

~~reacting the sample sulfonylhydrazone with a sulfonyl halide following reacting the sample with the sulfonylhydrazide; and~~

analyzing the reacted sample by mass spectrometry to detect the ketosteroid by detecting the sulfonyl halide derivative of the sulfonylhydrazone of the ketosteroid, wherein detection of the sulfonyl halide derivative of the sulfonylhydrazone indicates presence of the ketosteroid.
2. (Previously presented) The method of claim 1, wherein analyzing the sample by mass spectrometry comprises atmospheric pressure ionization.
3. (Previously presented) The method of claim 2, wherein atmospheric pressure ionization comprises positive ion mode electrospray ionization.
4. (Previously presented) The method of claim 1 further comprising separating the ketosteroid from other components in the sample by liquid chromatography.
5. (Original) The method of claim 4, wherein the liquid chromatography is high performance liquid chromatography (HPLC).
6. (Previously presented) The method of claim 4, wherein the ketosteroid is reacted with the sulfonylhydrazide prior to separating the ketosteroid by liquid chromatography.

7. (Previously presented) The method of claim 5, wherein separating the ketosteroid from other components in the sample by HPLC comprises reverse phase HPLC using a non-polar stationary phase.
8. (Previously presented) The method of claim 7 wherein reverse phase HPLC is performed using a methanol/water solvent.
9. (Previously presented) The method of claim 7, wherein the non-polar stationary phase is a C18 stationary phase.
10. (Previously presented) The method of claim 8, wherein HPLC is performed with gradient elution from 20:80 methanol/water to 80:20 methanol/water is used.
11. (Previously presented) The method of claim 10, wherein gradient elution is performed from 40:60 methanol water to 60:40 methanol water is used.
12. (Original) The method of claim 1 further comprising extracting the ketosteroid from the sample prior to reacting the sample with the sulfonylhydrazide to provide a concentrated sample for analysis.
13. (Previously presented) The method of claim 1, wherein the ketosteroid is an estrogen.
14. (Previously presented) The method of claim 13, wherein the ketosteroid is a catechol estrogen.
15. (Previously presented) The method of claim 1, wherein the sulfonylhydrazide is *p*-toluenesulfonylhydrazide.
16. (Cancelled).

17. (Previously presented) The method of claim 1, wherein the sulfonyl halide comprises



wherein X is Cl, Br, or I, and R is alkyl, substituted alkyl, aryl, or substituted aryl.

18. (Original) The method of claim 17, wherein R comprises lower alkyl.

19. (Withdrawn) A method for enhancing positive ion mode electrospray ionization efficiency of a carbonyl compound comprising reacting a carbonyl compound with a sulfonylhydrazide to form a sulfonylhydrazone of the carbonyl-containing compound that is efficiently ionized by electrospray ionization processes.

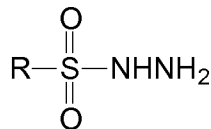
20. (Withdrawn) The method of claim 19, wherein the carbonyl-containing compound is a ketosteroid.

21. (Withdrawn) The method of claim 20, wherein the ketosteroid is selected from the group consisting of androgens, corticoids, estrogens, sterols, vitamin D metabolites, phytosteroids, neurosteroids and bile acids, and combinations thereof.

22. (Withdrawn) The method of claim 21, wherein the ketosteroid is an estrogen.

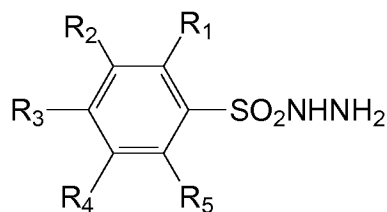
23. (Withdrawn) The method of claim 22, wherein the estrogen is a catechol estrogen.

24. (Withdrawn) The method of claim 19, wherein the sulfonylhydrazide comprises



wherein R is selected from the group consisting of alkyl, substituted alkyl, aryl, and substituted aryl.

25. (Withdrawn) The method of claim 24, wherein the sulfonylhydrazide comprises



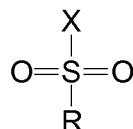
wherein R₁-R₅ are independently selected from the group consisting of hydrogen, C1-C5 alkyl, C1-C4 alkoxy, halogen, amino, nitro, hydroxyl, carbonyl, nitroso, cyano, and sulfonyl, and combinations thereof.

26. (Withdrawn) The method of claim 25, wherein the sulfonylhydrazide is *p*-toluenesulfonylhydrazide.

27. (Withdrawn) The method of claim 19 further comprising reacting the carbonyl compound with a sulfonyl halide after forming the sulfonylhydrazone.

28. (Withdrawn) The method of claim 27, wherein the sulfonyl halide comprises a sulfonyl chloride.

29. (Withdrawn) The method of claim 27, wherein the sulfonyl halide comprises



wherein X is Cl, Br, I, or any good leaving group, and R is alkyl, substituted alkyl, aryl, and substituted aryl.

30. (Withdrawn) A method for separating and detecting ketosteroids present in a biological sample, comprising:

extracting a ketosteroid from a biological sample to provide a concentrated sample of the ketosteroid;

reacting the concentrated sample of the ketosteroid with *p*-toluenesulfonylhydrazide to form a *p*-toluenesulfonylhydrazone derivative of the ketosteroid;

separating the *p*-toluenesulfonylhydrazone derivative of the ketosteroid from other components in the concentrated sample by reverse phase liquid chromatography;

detecting the *p*-toluenesulfonylhydrazone derivative of the ketosteroid by its API-MS signal to detect the ketosteroid in the sample.

31. (Withdrawn) The method of claim 30 further comprising reacting the *p*-toluenesulfonylhydrazone derivative of the ketosteroid with a sulfonyl halide to form a sulfonyl halide derivative of the *p*-toluenesulfonylhydrazone derivative of the ketosteroid, prior to separating the *p*-toluenesulfonylhydrazone derivative of the ketosteroid from other components.

32. (Withdrawn) The method of claim 30 further comprising adding a known amount of a deuterated analog of the ketosteroid to the biological sample prior to extracting to quantify the ketosteroid in the sample by comparison of API-MS signals from the ketosteroid and its deuterated analog.

33. (Withdrawn) The method of claim 30 wherein the biological sample is urine.

34. (Withdrawn) The method of claim 30 wherein the ketosteroid is an estrogen.

35. (Withdrawn) The method of claim 34 wherein the estrogen is a catechol estrogen.
36. (Withdrawn) The method of claim 30 wherein separating by liquid chromatography comprises separating by high performance liquid chromatography (HPLC).
37. (Withdrawn) The method of claim 36 wherein separating by HPLC comprises separating by reverse phase HPLC in a methanol/water mobile phase and a C18 stationary phase.
38. (Withdrawn) A kit for use in a method for detecting a ketosteroid in a sample by MS, the kit comprising in packaged combination:
a sulfonylhydrazide compound; and
a deuterated standard of the ketosteroid.
39. (Withdrawn) The kit of claim 38 further comprising a sulfonyl halide.
40. (Withdrawn) The kit of claim 38, wherein the sulfonylhydrazide compound comprises *p*-toluenesulfonylhydrazide.
41. (Withdrawn) The kit of claim 39, wherein the sulfonyl halide comprises sulfonyl chloride.
42. (Withdrawn) The kit of claim 38, wherein the ketosteroid is a catechol estrogen and the deuterated standard is a deuterated catechol estrogen.
43. (Withdrawn) A method for detecting an endogenous steroid in a sample, comprising:
reacting the sample with a carbonyl protecting reagent that reacts with a carbonyl group that may be present in the endogenous steroid to form a carbonyl derivative of the

endogenous steroid, and then reacting the sample with a hydroxyl protecting reagent that reacts with a hydroxyl group present in the endogenous steroid to form a hydroxyl derivative, wherein both reacting steps together provide a derivatized endogenous steroid; and

analyzing the reacted sample by mass spectrometry to detect the endogenous steroid if it is present by detecting the derivatized endogenous steroid.

44. (Withdrawn) The method of claim 43 further comprising separating the endogenous steroid from the reacted sample by liquid chromatography prior to analyzing the reacted sample.

45. (Withdrawn) The method of claim 43, wherein the carbonyl protecting reagent comprises a compound that forms an oxime derivative, a silyl derivative, an ketal/acetal, a hydrazone, or a Schiff's base derivative.

46. (Withdrawn) The method of claim 45, wherein the carbonyl protecting reagent comprises methoxyamine, ethoxyamine, carboxymethoxylamine, Girard's Reagent T, Giard's Reagent P, 6-ethoxy-2-benzothiazolesulfonamide, cystein, N'-(2-Thiazolyl) sulfanilamide, sulfisomidine, sulfadiazine, or p-toluenesulfohydrazide (TSH).

47. (Withdrawn) The method of claim 46, wherein the hydroxyl protecting reagent comprises a compound that forms a silyl derivative, an acyl derivative, a benzoyl derivative, an alkyl derivative, a dansyl derivative, or a nitrobenzofurazan derivative.

48. (Withdrawn) The method of claim 47, wherein the hydroxyl protecting reagent comprises nitrobenzopentafluorobenzoyl hydroxylamine, hydroxylamine, dabsyl chloride, dansyl chloride, 1-fluoro-2,4-dinitrobenzene, or 4-fluoro-3-nitrobenzofurazan.

49. (Withdrawn) The method of claim 43, wherein the carbonyl protecting reagent comprises a sulfonylhydrazide.

DOCKET NO.: NIHA-0249 (E-103-2002/0-US-03)
Application No.: 10/511,409
Office Action Dated: October 23, 2009

PATENT

50. (Withdrawn) The method of claim 43, wherein the hydroxyl protecting reagent comprises a sulfonyl halide.

51. (Withdrawn) The method of claim 43, wherein the carbonyl protecting reagent comprises a sulfonylhydrazide and the hydroxyl protecting reagent comprises a sulfonyl halide.